Blood Oxygen Depletion in Diving California Sea Lions: How Close to the Limit?

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LONG-TERM GOALS

The management and depletion of O_2 stores underlie the dive capacities of marine mammals and are fundamental to the concept of an aerobic dive limit (ADL, dive duration associated with the onset of post-dive blood accumulation). The ADL, which is often calculated (cADL) on the basis of total body O_2 stores and an estimated diving metabolic rate, has become an essential concept in the interpretation of diving behavior and foraging ecology (Kooyman and Ponganis 1998); however, the actual rate and magnitude of O_2 store depletion during dives has not been determined in any otariid. This project documented the rate and magnitude of blood O_2 store depletion during diving in California sea lions, and this information will be used to develop an experimental approach to assess the aerobic dive limit (ADL) and O_2 store depletion in this and other otariid species.

OBJECTIVES

The specific objectives of this project are: 1) Document venous and arterial P_{O2} profiles in lactating California sea lions while diving during foraging trips to sea, 2) Characterize the O_2-hemoglobin (Hb) dissociation curve of sea lion Hb, 3) Convert the P_{O2} profiles into % Hb saturation (S_{O2}) profiles with the dissociation curve, and then calculate the rate and magnitude of blood oxygen depletion, and 4) Refine vascular access techniques to allow future investigations of blood N_2 kinetics, changes in blood pH, P_{CO2}, P_{O2} and lactate during dives, and stress responses to captivity, training, and/or sound exposure.

APPROACH

Objectives 1 & 4: Backpack recorders measured blood P_{O2} during dives of foraging trips of lactating California sea lions on San Nicolas Island, CA. Females were anesthetized and instrumented with a P_{O2} logger, time depth recorder and radio tag, released, and recaptured after 1-4 foraging trips. With ultrasound guidance over the caudal back, a P_{O2} electrode and thermistor were inserted percutaneously into the vena cava (n = 11) or aorta (n = 2). (key personnel: B.I. McDonald, P.J. Ponganis, Navy Anesthesia resident)

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Objective 2: The O_2-Hb dissociation curve was determined at four pH values (7.5, 7.4, 7.3 & 7.2) from blood samples obtained from sea lions at Sea World's Rehabilitation Program (n = 7) and the National Marine Mammal Foundation (n=4) using the mixing technique. (B.I. McDonald)

Objective 3: The S_{O2} values during dives were obtained with application of the dissociation curve analysis to the P_{O2} data. These data allowed calculation of maximum and minimum blood O_2 content of a dive, net and rate of blood O_2 depletion and rate of O_2 content depletion, and net contribution of blood O_2 to metabolic rate during diving (ml O_2 kg^{-1} min^{-1}). (key personnel: B.I. McDonald, P.J. Ponganis)

WORK COMPLETED

We have completed all objectives and are in the process of writing up the results. Over the course of the project we captured, instrumented and recovered backpack P_{O2} recorders and Time Depth Recorders from 12 lactating California sea lions. P_{O2} electrodes and thermistors were successfully placed in the caudel gluteal vein using sterile techniques in the field in 10 of the females and PO_2 electrodes were successfully placed in an artery in 2 of the females. Processing and analysis of the P_{O2} data and dissociation curve analysis is complete. One paper was just published online, one paper is in prepreation, and currently temperature data are being analyzed.

RESULTS

<u>P_{O2} profiles:</u> We obtained 2619 venous P_{O2} profiles from 8 sea lions and 1273 arterial P_{O2} profiles from one sea lion (dives > 1 min in duration). Minimum venous P_{O2} was 5 mmHg in dives greater than 7 min and minimum arterial P_{O2} was 10 mmHg in a 4 min dive, consistent with extreme hypoxemic tolerance.

<u>Hb-O_2 dissociation curve:</u> The shape of the curve was typical of mammals; the P_{50} was 28 ± 2 mmHg (n=11). The Bohr effect was unremarkable (-0.54).

<u>S_{O2} profiles and O_2 depletion:</u> Arterial and venous minimum S_{O2} were routinely greater than 50% and variable during routine shallow dives (Fig 1, 2). In deep dives greater than 4 min in duration, minimum venous S_{O2} reached values below 10%, and in dives over 6 min, were as low as 1% (Fig 1, 3A). The latter are consistent with near complete venous blood O_2 depletion (Fig 3). In a 6-min dive, such depletion of venous blood O_2 would contribute 2.7 ml O_2 kg^{-1} min^{-1} to dive metabolic rate.

During deep dive bouts, both arterial and venous S_{O2} increased during ascent (Fig 1, 3). The increase in arterial and venous S_{O2} during ascent from deep dives suggests that re-expansion of collapsed lungs and resumption of gas exchange during the "ascent tachycardia" allows for lung-to-blood O_2 transfer.

Arterial P_{O2} and S_{O2} depletion patterns of serial deep dives differed from shallow dives and isolated deep dives with abrupt declines and increases at about 200m depth during descent and ascent, respectively (Fig 3B). We suggest this is due to cessation of gas exchange at depth and that an O_2 reservoir in the collapsed lungs serves to supplement blood O_2 levels during ascent.

<u>Conclusions:</u> Although California sea lions have extreme hypoxemic tolerance and the blood O_2 store contributes significantly to metabolic rate during a dive, the lung O_2 store may play a greater role than previously suspected in serial deep dives (arterial S_{O2} is maintained). In addition, given these findings,

we hypothesize that the most significant limit to dive performance and aerobic dive capacity may be depletion of the muscle O_2 store.

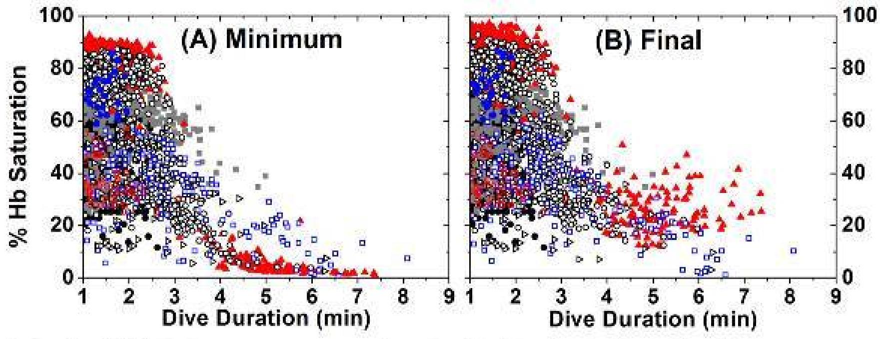

Figure 1. (A) Minimum venous S_{O2} & (B) End-of-dive (final) venous S_{O2} vs. dive duration in 8 sea lions. As dive duration increases, minimum and final S_{O2} decrease, but are variable in short duration dives. In dives > 4 min, minimum S_{O2} is often < 10%. At the end of long deep dives, final S_{O2} is > minimum S_{O2}, suggesting resumption of gas exchange and/or increased arterio-venous shunting.

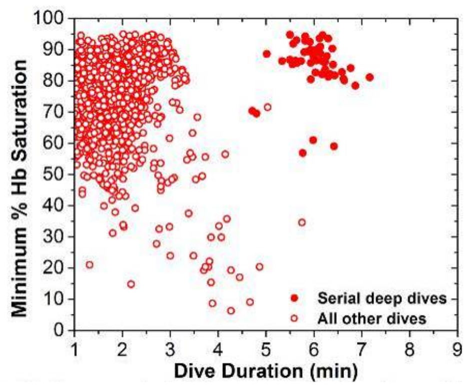

Figure 2. Minimum arterial SO2 vs. dive duration. Minimum S_{O2} is highly variable in short dives, but decreases with duration in most dives > 3 min. In serial deep dives minimum S_{O2} is consistently high suggesting different O_2 management strategies.

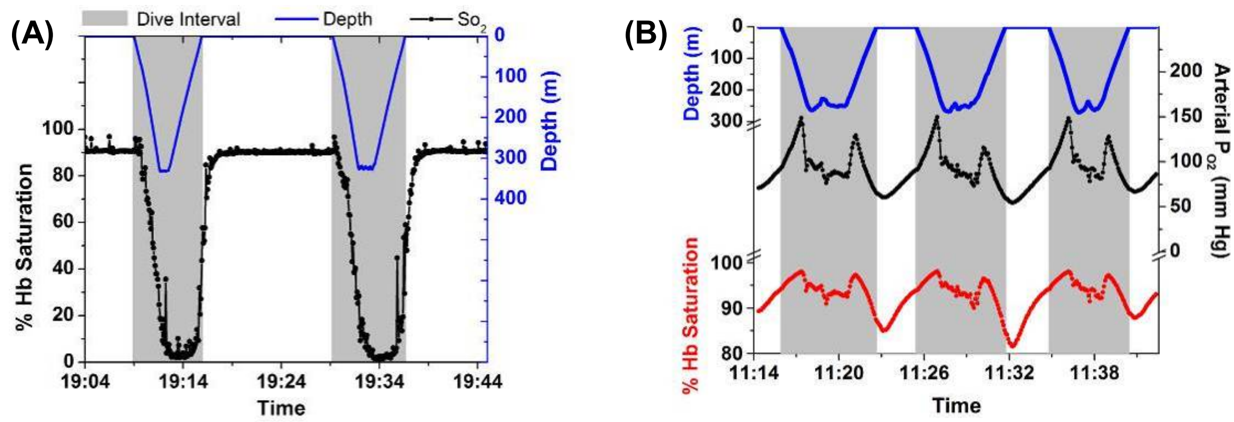

Figure 3 (A) Venous S_{O2} profiles and (B) Arterial P_{O2} and S_{O2} profiles from serial deep dives. (A) During the last minute of the dive venous S_{O2} increased suggesting resumptionof gas exchange or arterio-venous shunting. (B) The abrupt declines and increases in arterial P_{O2} and S_{O2} occur at approximately 200-m depth during descent and ascent, respectively, consitent with cessation of gas exchange below that depth.

IMPACT/APPLICATIONS

This project directly assesses blood O_2 depletion in diving California sea lions and develops catheterization techniques (including ultrasound guidance) for these animals. These findings thus extend beyond just the foraging ecology of sea lions, and are relevant to many important topics in diving physiology including: a) the role of the diving bradycardia in gas exchange, reduction of organ blood flow, and management of O_2 stores (Davis et al. 1983; Scholander 1940), b) the significance of myoglobin, hydrodynamics, and locomotory swim patterns in depletion of the muscle O_2 store and the ADL (Noren et al. 2001; Williams et al. 2000), c) time partitioning of energetic demands into different dive types or surface intervals in order to conserve O_2 stores during diving (Sparling et al. 2007; Williams et al. 2004), d) the role of neuroglobin and cytoglobin in hypoxemic tolerance (Williams et al. 2008), e) the role of free radicals and oxidative stress in diving mammals (Elsner et al. 1998) and f) the basic assumptions of many recent computer models of the uptake and distribution of N_2 during diving (Fahlman et al. 2009; Houser et al. 2001).

Refinement of vascular access techniques in this project also provides the groundwork to apply this technology to other otariids, and possibly other marine mammals, including dolphins. Development of such approaches is especially pertinent to the question of nitrogen uptake and its role in beaked whale strandings after exposure to naval sonar (Cox 2006). And, in fact, given that access to cetaceans for physiological investigations is so difficult, the sea lion, with a lung O_2 store as significant as that in cetaceans (Ponganis et al. 2003), may be an appropriate surrogate model for further investigation of blood N_2 uptake during diving.

RELATED PROJECTS

Deep-diving California sea lions: Are they pushing their physiological limit? (Award #: N000141210633) is a continuation of this project. The research questions we are addressing in the new project are building upon the results from this study.

REFERENCES

Cox, T.M., et al. 2006. Understanding the impacts of anthropogenic sound on beaked whales. Journal of Cetacean Research Management 7: 177-187.

Davis, R.W., M.A. Castellini, G.L. Kooyman and R. Maue. 1983. Renal GFR and hepatic blood flow during voluntary diving in Weddell seals. American Journal of Physiology 245: 743-748.

Elsner, R., S. Oyasaetr, R. Almaas and O.D. Sauagstad. 1998. Diving seals, ischemia-reperfusion, and oxygen radicals. Comparative Biochemistry and Physiology A 119: 975-980.

Fahlman, A., S.K. Hooker, A. Olszowka, B.L. Bostrom and D.R. Jones. 2009. Estimating the effect of lung collapse and pulmonary shunt on gas exchange during breath-hold diving: The Scholander and Kooyman legacy. Respiration Physiology and Neurobiology 165: 28-39.

Houser, D.S., R. Howard and S. Ridgway. 2001. Can diving-induced tissue nitrogen supersaturation increase the chance of acoustically driven bubble growth in marine mammals. Journal of Theoretical Biology 213: 183-195.

Kooyman, G.L., and P.J. Ponganis. 1998. The physiological basis of diving to depth: birds and mammals. Annual Review of Physiology 60: 19-32.

Nicol, S. 1991. Respiratory properties of the blood of the little blue penguin Eudyptula minor. Comparative Biochemistry and Physiology A 98: 17-22.

Noren, S.R., T.M. Williams, D.A. Pabst, W.A. McLellan and J.L. Dearolf. 2001. Development of diving in marine endotherms: preparing the skeletal muscles of dolphins, penguins, and seals for activity during submergence. Journal of Comparative Physiology B 171: 127-134.

Ponganis, P.J., G.L. Kooyman and S.H. Ridgway. 2003. Comparative Diving Physiology. pp. 211-226 in A.O. Brubakk and T.S. Neuman, eds. Bennett and Elliott's Physiology and Medicine of Diving. Saunders, Edinburgh.

Scheid, P., and M. Meyer. 1978. Mixing technique for study of oxygen-hemoglobin equilibrium: a critical evaluation. Journal of Applied Physiology 45: 812-822.

Scholander, P.F. 1940. Experimental investigations on the respiratory function in diving mammals and birds. Hvalradets skrifter 22: 1-131.

Sparling, C.E., M.A. Fedak and D. Thompson. 2007. Eat now, pay later? Evidence of deferred food processing costs in diving seals. Biology Letters 3: 94-98.

Weise, M.J., and D.P. Costa. 2007. Total body oxygen stores and physiological diving capacity of California sea lions as a function of sex and age. Journal of Experimental Biology 210: 278-289.

Williams, T.M., R.W. Davis, L.A. Fuiman, J. Francis, B.J. Le Boeuf, M. Horning, J. Calambokidis and D.A. Croll. 2000. Sink or Swim: Strategies for Cost-Efficient Diving by Marine Mammals. Science 288: 133-136.

Williams, T.M., L.A. Fuiman, M. Horning and R.W. Davis. 2004. The cost of foraging by a marine predator, the Weddell seal Leptonychotes weddellii: pricing by the stroke. Journal of Experimental Biology 207: 973-982.

Williams, T.M., M. Zavanelli, M.A. Miller, R.A. Goldbeck, M. Morledge, D. Casper, D.A. Pabst, W. McClellan, L.P. Cantin and D.S. Kliger. 2008. Running, swimming, and diving modifies neuroprotecting globins in the mammalian brain. Proceedings of the Royal Society B 275: 751-758.

PUBLICATIONS

McDonald, B.I. and P.J. Ponganis. (published online Sept. 19, 2012). Lung collapse in the diving sea lion: hold the nitrogen and save the oxygen. Biology Letters.

HONORS/AWARDS/PRIZES

Birgitte I. McDonald, Scripps Institution of Oceanography, Young Scientist Award Finalist, Society of Experimental Biology.